Progress on the hybridization of cultivated lentil *Lens culinaris* Medik. and wild species *Lens tomentosus* Ladizinsky

Devini de Silva *, Chandra Bandara and Albert Vandenberg
Crop Development Centre, University of Saskatchewan, Saskatoon, SK, S7N 5A8

*Presenter (nad339@mail.usask.ca)

INTRODUCTION

1. Wild lentil species have natural resistance to many pathogens, for example, anthracnose (*Colletotrichum* spp.), ascochyta blight (*Ascochyta lentis*), stemphylium blight (*Stemphylium botryosum*) and *Orobanche* spp. They may also have traits of interest for improvements in tolerance to abiotic stresses.

2. Previous attempts to incorporate some desirable genes for long term crop improvement by hybridizing cultivated lentil (*L. culinaris* Medik.*)* with wild lentil species (*L. tomentosus* Ladizinsky*)* have failed to produce viable seeds (Tullu et al. 2011).

3. Embryo rescue, an alternative technique employed for producing healthy embryos following hybridization, is time consuming and requires controlled growing environments and highly skilled technical personnel have been tried with limited success (Fratini and Ruiz, 2006).

OBJECTIVES

1. To produce F_1 and F_2 viable seeds through interspecific hybridization of *L. culinaris* and *L. tomentosus* by employing conventional crossing techniques, without using embryo rescue methods.

2. To evaluate these hybrids phenotypically and to characterize them through genotyping to establish a strong foundation to broaden the genetic base of lentil breeding programs.

MATERIALS AND METHODS

1. Lentil cultivars 'Indianhead' and 'CDC Redberry' were used as pollen recipients, and L. *tomentosus* IG 72613 and IG 72805 were used as pollen donors.

2. This study was conducted in a controlled environment (16 h/8 h day/night 21°C/15°C) of the University of Saskatchewan during period of 2015 Fall to 2016 Fall.

3. Three scarified seeds of each parental genotype were planted in 4-L pot filled with SunGro #4, and two weeks after emergence, seedlings were thinned to one healthy seedling per pot. Optimal cultural practices, including fertilization, pest and disease control, were used to maintain healthy plants.

4. At flowering, pollination was carried out by applying pollen from donors to the stigma of recipient flowers after removal of sepals, petals and stamens. Crossing tools were sterilized with 95% ethanol to minimize cross contamination (Figs. 1 and 2).

5. Seeds of F_1 generation from successful crosses were harvested at maturity.

6. The F_1 seeds were planted and seedlings were grown under aforementioned growing conditions to produce F_2 generation.

7. Fifty seeds of F_2 generation were randomly selected and seed weights were determined.

Fig. 1. Emasculated lentil bud

Fig. 2. Emasculated lentil bud after pollination

RESULTS AND DISCUSSION

1. F_1 seeds were successfully produced from interspecific hybridization of 'Indianhead' (yellow cotyledon) and 'IG 72613' (red cotyledon) and confirmed by the red cotyledon color of crossed seeds. Cross # 8292 was assigned to the F_1 seeds (Fig. 3).

2. F_1 seeds of 'CDC Redberry' (red cotyledon, white flowers) X 'IG 72805' (red cotyledon, purple flowers) were assigned with cross # 8272. Successful hybridization was confirmed based on the flower color (light purple) of F_1 plants as well as F_2 seed coat color (Fig. 4).

3. F_1 plants of 8292 and 8272 had increased pubescence, characteristics of the male parent.

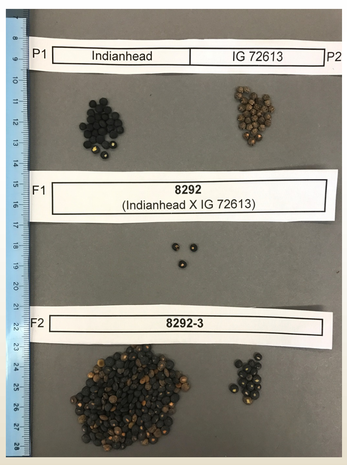

Fig. 3. Parent, F_1 and F_2 seeds of 'Indianhead' and IG 72613

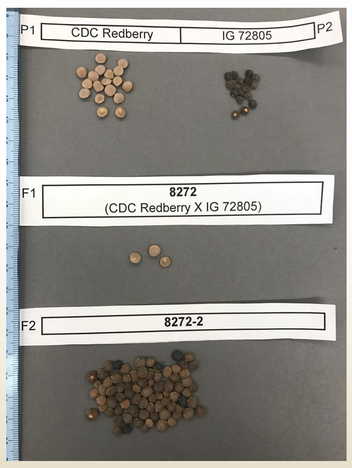

Fig. 4. Parent, F_1 and F_2 seeds of 'CDC Redberry' and 'IG 72805'

RESULTS AND DISCUSSION CONT'D

4. F_1 plants of crosses 8292 and 8272 flowered earlier than their respective parents (Fig. 5)

Fig. 5. Growth and development at F_1 flowering stage for parents and F_1 plant of cross 8292 seeded at the same time

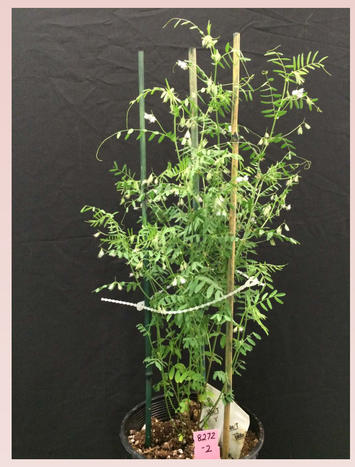

Fig. 6. 8272 F_1 plant at flowering and pod setting

5. Seed weight of F_2 seeds of 8292 and 8272 shows transgressive segregation (Figs. 7 & 8)

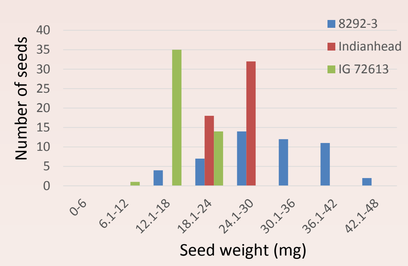

Fig. 7. Frequency distribution of 50 randomly selected seeds of parents and F_2 of cross 8292

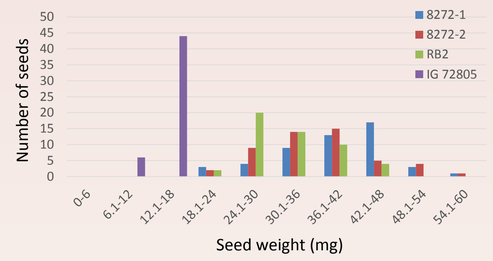

Fig. 8. Frequency distribution of 50 randomly selected seeds of parents and F_2 of cross 8272

CONCLUSIONS

1. Introgression of alleles from *L. tomentosus* into cultivated lentil *L. culinaris* can be achieved by conventional crossing techniques without embryo rescue.

2. Genetic improvement, productivity and quality of lentil can be enhanced by expediting the expansion of genetic diversity available in the primary gene pool of the cultivated lentil.

FUTURE WORK

1. Interspecific crossing between *L. culinaris* with *L. lamottei* and *L.nigricans* will be attempted using similar techniques.

2. An interspecific RIL population of 8272 is being developed for further studies.

3. Pubescence phenotypes of individual plants in the F_2 populations were characterized and recorded for future genetic analyses and introgression studies.

ACKNOWLEDGEMENTS

We would like to thank:

-NSERC, SPG, ADF and the University of Saskatchewan for financial support

-Yong Liu for his assistance

REFERENCES

1. Fratini, R and Ruiz, M.L. 2006. Interspecific hybridization in the genus *Lens* applying *in vitro* embryo rescue. Euphytica, 150:271-280.

2. Tullu, A., Diederichsen, A., Suvorova, G. and Vandenberg, A. 2011. Genetic and genomic resources of lentil: status, use and prospects. Plant Genetic Resources: Characterization and Utilization: 9 (1): 19-29.

3. Wong, M.M.L., Gujaria-Verma, N., Yuan, H. Y., Ramsay, L., Caron, C., Diapari , M., Vandenberg, A. and Bett, K. 2015. Classification and characterization of species within the genus *Lens* using genotyping-by-sequencing (GBS) PLoS ONE 10(3): e0122025. doi:10.1371/ journal.pone.0122025.